**Nuclei Isolation Method (frozen human brain tissue)**

**Combined Method from Krishnaswami et al 2016 Nature Protocol (https://www.nature.com/articles/nprot.2016.015), and 10x Genomics**

Pre-cool everything

Nuclease-free reagents

* Make NIM1, NIM2 and Homogenisation Buffer (HB), 29% and 50% Iodixanol, from Nature Protocol.
* Put 800ul HB in 2ml douncer. Add tissue (50-250mg).
* 5 strokes with loose A pestle, wash pestle with 100ul HB.
* 10-15 strokes with tight B pestle, wash pestle with 100ul HB.
* Use 200ul tip to remove liquid + tissue from douncer and filter through facs filter tube.
* Transfer to ependorf tube
* Centrifuge 1000g for 8min
* Remove (most) supernatant and gently resuspend in 250ul HB.
* Filter through facs filter tube.
* Add 500ul 29% iodixanol to a new ependorf.
* Add 250ul 50% iodixanol to the nuclei and mix gently
* Slowly layer the nuclei/sucrose mix ontop of the 29% sucrose in the ependorf.
* Centrifuge 13000g for 40min [time from 10x Protocol]
* Remove supernatant and all myelin (on top).
* Wash with 1ml PBS + 1% BSA + 0.2U/ul RNAse inhibitor. Filter through facs tube. Centrifuge 500g for 5min.
* Wash again.
* Take 10ul nuclei aliquot, add 1ul Dapi, view on microscope to assess debris. Repeat wash step if necessary.
* Take 1ml of nuclei on ice to Genomics lab to count on Luna and load on 10X chip.